MicroRNAs in the skin; role in development, homeostasis, and regeneration

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Abstract

The skin is the largest organ of the integumentary system and possesses a vast number of functions. Due to the distinct layers of the skin and the variety of cells that populate each, a tightly regulated network of molecular signals control development and regeneration, whether due to programmed cell termination or injury. MicroRNAs (miRs) are a relatively recent discovery; they are a class of small non-coding RNAs that possess a multitude of biological functions due to their ability to regulate gene expression *via* post-transcriptional gene silencing. Of interest, is that a plethora of data demonstrates that a number of miRs are highly expressed within the skin, and are evidently key regulators of numerous vital processes to maintain non-aberrant functioning. Recently, miRs have been targeted as therapeutic interventions due to the ability of synthetic 'antagomiRs' to downregulate abnormal miR expression, thereby potentiating wound healing and attenuating fibrotic processes which can contribute to disease such as systemic sclerosis (SSc). This review will provide an introduction to the structure and function of the skin and miR biogenesis, before summarising the literature pertaining to the role of miRs. Finally, miR therapies will also be discussed, highlighting important future areas of research.

Keywords: microRNA, skin, wound, development, homeostasis, regeneration.

1. Introduction

1.1 Skin

The skin is the largest and one of the most varied organs of the body, and possesses a vast number of functions including thermoregulation, protection from external stimuli and pathogens, as well as water resistance. Composed of three layers, the skin acts as a protective barrier against environmental exposures. The top layer, the epidermis, is composed of proliferating basal and differentiated suprabasal keratinocytes, in addition to melanocytes, Langerhans and Merkel cells.

Keratinocytes are the most abundant cell type located in the epidermis and possess a number of immune functions. For example, they exert a protective role *via* secretion of chemokines upon invasion of a pathogenic threat (1). Production of a broad range cytokines, with both pro- and anti-inflammatory functions also occurs (2). Expression of various Toll-like receptors (TLRs) contributes to the defence of the skin against antimicrobial pathogens (3). In addition to their immune functions, they also secrete keratin, a key structural protein, and finally become the cornified layer once the cells flatten. Melanoblasts proliferate and colonise in the epidermis as melanocytes. Located in the basal layer, these cells produce melanin, the pigment found in skin, hair and eyes that protects against UV radiation (4). Keratinocytes stimulate the proliferation and differentiation of melanocytes, in addition to melanogenesis. Epidermal and follicular homeostasis, therefore, is maintained largely by interactions between these two cell types (4). Langerhans cells are dendritic cells that process microbial antigens and are able to induce T cell differentiation and polarisation (5), in addition to activation of regulatory T cells (6). In contrast, Merkel cells which are located in the basal epidermis, function as mechanoreceptors (7). Homeostasis of the epidermis is maintained by three distinct classes of stem cells; bulge stem cells of located in the hair follicle, interfollicular stem cells, and sebaceous gland stem cells.

The middle layer, the dermis, is populated by macrophages, lymphocytes, mast cells, and dendritic cells, in addition to fibroblasts which provide structural strength through production of collagen and elastin fibers and other extracellular matrix proteins (8). Meissner's and Pacinian corpuscles, in addition to nerve fibres and free endings, detect touch, pressure, and pain (9), while sweat and sebaceous glands regulate temperature. Not only do capillary networks in the dermis provide the epidermis with oxygen and nutrients (8), but dermal stem cells are able to differentiate into functional cells in the epidermis, highlighting an interdependence between the two layers (10).

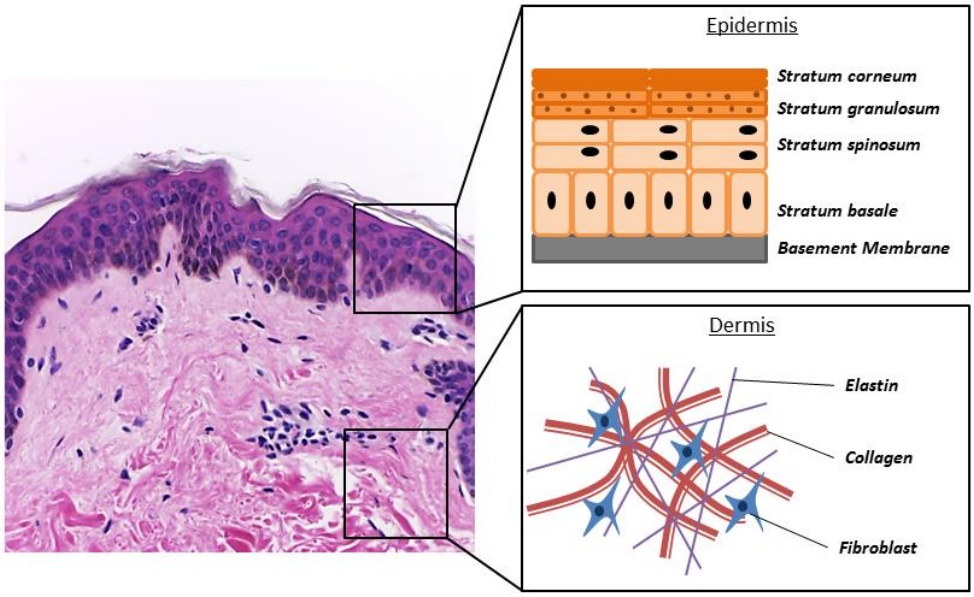

Figure 1 – Structure of the epidermis and dermis.

The hypodermis is primarily composed of adipocytes, and is also involved in thermoregulation *via* its insulative properties, while production of vitamin D also occurs in this subcutaneous layer.

The large number of cell types and functional diversity of the skin necessitate tightly regulated molecular signals in order to deter aberrant growth and maintain homeostasis. MicroRNAs (miRs) are one group of molecules that aid in such mechanisms.

1.2 MicroRNA Biogenesis and Function

MiRs are small non-coding RNA molecules, typically ~22 nucleotides (nt) long, involved in RNA silencing and post-transcriptional regulation of gene expression by binding to the 3'UTR of its targets; target recognition occurs via a 6-8 nt site that matches the miR seed region. Each miR is capable of repressing hundreds of genes, and each gene can be targeted by multiple miRs, making it a powerful system for the fine-tuning of gene expression.

MiR biogenesis is similar to protein-coding genes in that upon transcription factor binding, RNA polymerase II transcribes the miR gene, followed by Drosha-mediated cleavage of the primary miR transcript resulting in a number of precursor miRs (pre-miR). A 2 nt overhang at the 3' end exists as a binding site for Exportin-5 which causes translocation to the cytoplasm. Dicer then processes the pre-miR into a mature miR strand which is incorporated into the RNA-induced silencing complex (RISC), which in turn, inhibits translation (see figure 2). Reduction in the abundance of the target mRNA may also occur *via* RNA cleavage or decapping and deadenylation. The RISC can also silence genes *via* formation of heterochromatin at the genomic level (11). A number of miRs are highly expressed in the skin, and possess a plethora of important functions with regard to development, homeostasis, and regeneration. The following sections serve to summarise the current literature within these areas. In addition,

chemically synthesized miRs are beginning to be trialled and used as therapeutic treatments for a number of diseases, and will also be discussed.

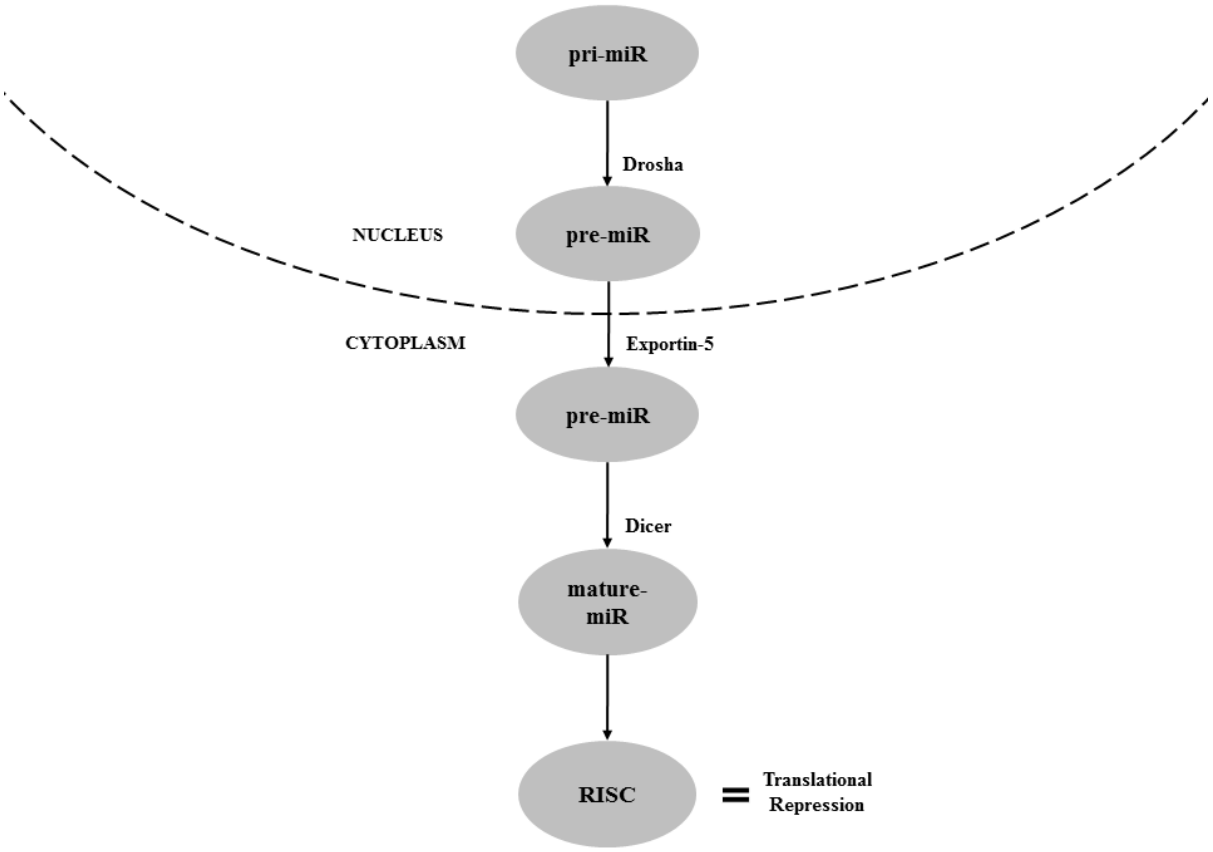

Figure 2 – Simplified microRNA biogenesis and incorporation into the RNA-induced silencing complex pathway.

2. Skin Development and Homeostasis: Role of MicroRNAs

During embryonic development, gastrulation occurs whereby the single-layered blastula transforms into the tri-layered gastrula. Epidermal progenitor cells of the outer layer, the ectoderm, cause hair follicles, sebaceous glands and the epidermis itself to grow. Mesenchymal cells begin to infiltrate the skin and in combination with the basal epidermal layer, begin to direct extracellular matrix proteins and growth factors. Once the formation of new keratinocyte layers (stratification) and differentiation of their properties is complete, the epidermis consists of distinct layers: a cornified layer (*stratum corneum*), a translucent layer (*stratum lucidum*), a granular layer (*stratum granulosum*), a spinous layer (*stratum spinosum*), and a basal layer (*stratum basale*). The inner layer of basal cells proliferate, while suprabasal cells terminally differentiate. Hair follicle morphogenesis occurs *via* interactions between epidermal keratinocytes and dermal fibroblasts. Primarily, the mesenchyme coordinates keratinocyte proliferation. The follicle continues to develop downwards, whereby differentiation results in the hair shaft and sebaceous gland. The epidermis, therefore, is composed of the interfollicular epidermis and a pilosebaceous unit which contains a hair follicle.

Epidermal homeostasis is maintained by three distinct pools of stem cells located in the interfollicular epidermis, the bulge (a region located at the bottom of the hair follicle outer root sheath), and the sebaceous gland. Stem cells continuously self-renew and differentiate into various cell lineages, which is an essential process in order to replenish cells such as keratinocytes that die due to injury or programmed termination. Skin barrier renewal *via* spinous transition of basal cells in mature skin occurs concomitantly with changes to gene expression, for example, Keratin5/14 (*K5/K14*) are downregulated, and Keratin1/10 (*K1/K10*) are upregulated (12).

The molecular signals that control the development of the skin are numerous and complex, with a clear role for miRs as key regulators of a variety of processes crucial to epidermal and follicular development and homeostasis. The importance of miRs in skin morphogenesis has been shown by progenitor cell *Dicer* knockdown in an embryonic mouse skin model in which hair germ cells evaginated towards the epidermis, instead of invaginating towards the dermis as usual (13). Interestingly, lower *Dicer* expression has been observed in mouse embryonic epidermis than the hair follicle, suggesting differential epigenetic regulation even between two conjoining structures (14).

The functional consequence of *Dicer* knockdown is also significantly different between the epidermis and hair follicle; one week after birth, epidermal proliferation and apoptosis was not significantly altered, however, elevated apoptosis and attenuated cell proliferation inhibited follicular development (13, 14). In support of the postulation that differential epigenetic signals regulate development of the epidermis and hair follicle, the *miR-19/20* and *miR-200* families are highly expressed in the former, while conversely, the *miR-199* family is exclusively expressed in the latter (13).

The transcription factor p63 is essential in epidermal development, as demonstrated by the death of p63-deficient mice immediately following birth (15), likely as it is important in maintaining stem cell proliferation (16). The Notch pathway is a regulator of interfollicular differentiation and active primarily in suprabasal cells (17), while inhibition of this pathway results in a lack of skin barrier development (18). In addition, the MAPK pathway regulates

epidermal proliferation and differentiation as evidenced by Mek1/2 deletion which results in underdevelopment of the skin (19). Conversely, over-activation of epidermal growth factor (EGF) signalling, involved in the MAPK pathway, causes augmented proliferation and epidermal tumour growth (20). Consistently, attenuated interfollicular proliferation occurred as a consequence of EGF downregulation.

MiR-203 is expressed in both human adult and foetal skin, although expression was not detectable until 17 weeks (21). Evidence suggests that miR-203 is an important regulator of keratinocyte differentiation; Protein Kinase C (PKC) inhibition blocked *miR-203* expression following calcium-induced differentiation, while overexpression of *miR-203* significantly augmented differentiation (22). These data demonstrate that PKC pathway activation is essential for *miR-203* upregulation, and thus, keratinocyte differentiation. Furthermore, miR-203 appears to promote epidermal differentiation *via* decreasing proliferation. Given that p63 regulates stem cell maintenance, miR-203-induced *p63* repression is a plausible mechanism by which miR-203 promotes differentiation (23). *SOCS3* is expressed basally in both human foetal and adult skin, and is also directly targeted by miR-203 (21), further supporting the role of this miR in skin development.

MiR-34a, -34c, -574-3p, and -720 also appear to be regulated by p63. Inhibition of *miR-34a* and *-34c* restored keratinocyte cell cycle progression, while knockdown of *p63* augmented both of these miRs concomitantly with inhibition of cell cycle regulators (24). p63 and iASPP (inhibitor of apoptosis stimulating protein of p53) are involved in an autoregulatory feedback loop; iASPP itself negatively regulates *miR-574-3p* and *miR-720*, which could, in turn, be negatively regulating *p63*. Depletion of iASPP in keratinocytes promotes differentiation concomitantly with an overexpression of these two miRs. Furthermore, *miR-574-3p* and *miR-720* expression in these cells correlate with stratification pattern (25), thereby supporting their role in epidermal differentiation, plausibly *via* downregulation of *p63*.

Another miR-200 family member, *miR-205*, exhibits one of the highest levels of expression in skin stem cells. Significantly attenuated neonatal epidermal and follicular development occur as a consequence of *miR-205* knockdown, resulting in thinner skin (26). Phosphorylated Protein Kinase B (pAKT), previously shown to be involved in skin stem cell renewal and proliferation (27), was reported to be downregulated in the knockout model, as miR-205 directly targets and represses negative regulators of PI3K signalling. Proliferation, therefore, of skin stem cells appears to occur *via* miR-205-mediated mechanisms which maintain pAKT.

In addition to *p63* expression, interfollicular, sebaceous gland, and hair follicle stem cells also express *K5* and *K14*. Maintenance of *miR-125b* in cells expressing *K14* was reported to sustain stemness, resulting in aberrant skin morphogenesis in the form of a thickened epidermis, lack of hair follicle development, and enlarged sebaceous glands, plausibly *via* an attenuation of Blimp1 protein level. The data suggest that this dysregulated follicular development is due to an increased number of early bulge cell divisions in the outer root sheath, restricting cell lineage commitment. Interestingly however, upon restoration of *miR-125b* to normal levels, these effects were reversed (28). MiR-125b does not, however, influence stem cell maintenance or activation, suggesting that the function of this miR is to primarily enhance stem cell renewal. Overall, these results indicate that miR-125b possesses the ability to repress stem cell differentiation, which appears logical given that *miR-125b* expression is low in early stem cell progeny.

In terms of hair follicle morphogenesis, the Wnt/β-catenin pathway is an important regulator of hair follicle specification; during development, augmentation of β-catenin results in failed hair follicle differentiation (29), while similarly, *Runx1* expression is also essential for follicular differentiation (30). Furthermore, Bone morphogenetic protein (BMP) ligands and antagonists are key, as evidenced by deletion of the BMP antagonist Noggin which attenuates development of the hair follicle (31). MiR-214 targets β-catenin; when overexpressed in keratinocytes, miR-214 inhibits proliferation and consequently hair follicle development due to attenuation of β-catenin and Lef-1. MiR-214, therefore, is a key regulator of Wnt signalling, which itself is key in follicular morphogenesis (32).

Smad proteins, which are signal transducers of the TGF signalling pathway, are also involved in follicular development *via* mediation of BMP signalling and inhibition of Wnt/ β-catenin signalling (33). Interestingly, Smads also regulate miR expression through transcriptional and post-transcriptional mechanisms (34), further supporting the importance of the TGF- β superfamily and miRs in the skinBMPs are expressed in the epidermis and mesenchyme, and are involved in skin development and tissue remodelling (35); BMP4 treatment resulted in significant inhibition of *miR-21* expression in primary mouse keratinocytes. Similarly, overexpression of the BMP antagonist *Noggin* significantly augments *miR-21* in a transgenic mouse model (36). Furthermore, the BMP4-mediated inhibition of cell proliferation and migration was prevented by miR-21. Given that *miR-21* is consistently overexpressed in a variety of tumour types, these findings suggest a mechanism by which the BMP pathway inhibits miR-21 in order to maintain normal function of the skin.

Embryonic RUNX1 deletion in the epidermis causes delayed hair follicle regeneration due to failure of bulge stem cell proliferation (37). In contrast to epidermal development whereby elevated β-catenin leads to a reduction in hair follicle differentiation, during adulthood, high levels of β-catenin cause abnormal growth of hair follicles (29). The aforementioned BMP pathway mediates bulge stem cell quiescence in adult mice, and as *BMP* expression reduces, bulge stem cells activate and augment hair follicle regeneration (38).

In terms of dermal homeostasis, *miR-145* is highly expressed in dermal fibroblasts, however, expression was reported to be significantly downregulated during reprogramming to pluripotent stem cells. Inhibition of *miR-145* resulted in an elevation in epithelial markers, and a reduction in mesenchymal markers, suggesting mesenchymal to epithelial transitioning, a key process in reprogramming of somatic cells to induced pluripotent stem cells. It seems clear, therefore, that the reduction in miR-145 significantly contributes to homeostatic control of the dermis *via* a switch from dermal fibroblasts to stem cells (39), which themselves can be programmed to a different cell type.

The literature discussed thus far demonstrates that a large number of miRs exert significant regulatory effect on the fine tuning of a variety of signalling pathways involved in epidermal and dermal development and homeostasis.

Table 1 – Summary of miRs involved in skin development and homeostasis.

miR	Cell/Model	Role
203.	Foetal/adult skin (human) (22).	Regulator of keratinocyte differentiation.
34a/34c.	Keratinocytes (mouse) (24).	p63-regulated repression of -34a/34c controls cell cycle progression.
574-3p/720.	Neonatal keratinocytes (human) (25).	Negatively regulated by iASPP*. Depletion of iASPP = miR expression and cell differentiation
205.	Mouse models (26).	Neonatal epidermal and follicular development.
125b.	Mouse models (28).	Enhances stem cell renewal and inhibits stem cell differentiation.
214.	Mouse models/keratinocytes (32).	Inhibits follicular development *via* targeting β-catenin.
21.	Mouse models/keratinocytes (36).	Inhibits effects of BMP4* on cell proliferation/migration, leading to aberrant growth.
145.	Human dermal fibroblasts (39).	Downregulated during reprogramming of somatic cells into pluripotent stem cells

*iASPP = inhibitor of apoptosis stimulating protein of p53; BMP4 = bone morphogenic protein 4.

3. MicroRNAs in Wound Healing and Skin Regeneration

The underlying molecular mechanisms that mediate the development and homeostasis of the skin that have been discussed thus far all refer to processes that occur in healthy, undamaged skin. However, when injury occurs, differing signals, including miRs, coordinate the many stages of skin regeneration. The principal stage, haemostasis, is characterised by vasoconstriction which reduces blood flow, and platelet degranulation which activates fibrin, a protein involved in clotting. The second phase involves inflammation, whereby neutrophils immediately migrate to the wound and phagocytose pathogens and tissue debris. Monocyte-derived macrophages cause efferocytosis which aids in the resolution of the acute inflammatory response. Proliferation then occurs, which is characterised by a number of processes which assist in the formation of new tissue; several cell types, in particular keratinocytes, fibroblasts, and endothelial cells, coordinate re-epithelialisation, collagen deposition, angiogenesis and granulation tissue formation. Finally, the remodelling phase involves full formation of the epidermal barrier primarily *via* extracellular matrix protein synthesis and apoptosis of immune cells. Scar formation may also occur during this phase, thought to be a consequence of aberrant inflammation, re-epithelialisation, and collagen deposition.

3.1. Haemostasis

MiR-143-145 appears to be essential during haemostasis, as evidenced by attenuated vasoconstriction, a critical event during the early stages of wound healing, and impaired vascular smooth muscle cell (VSMC) differentiation in *miR-143-145* knockout animals when compared with age-matched wildtype controls (40). Platelet-derive growth factor (PDGF) induces *miR-15b*, which is essential for VSMC proliferation (41). This mimics previous findings which showed that PDGF elicited *miR-221* upregulation concomitantly with VSMC proliferation *via* downregulation of *c-Kit* (42).

Fibrinogen, the precursor to fibrin which causes clots at the site of skin injury, is regulated by miRs with attenuation of the *FGB* transcript occurring due to *miR-409-3p* overexpression. MiR-29 reduced the levels of all three fibrinogen transcripts, *FGA*, *FGB*, and *FGG* (43).

Each of the aforementioned stages do not necessarily occur in isolation, as evidenced by miR regulation of overlapping haemostatic and inflammatory mechanisms. The pleiotropic cytokine IL-6 is a significant inducer of fibrinogen synthesis (44), which itself is regulated by the transcription factor STAT3. Expression of the *miR-17.92* family transcripts, *miR-18a*, *miR-19a*, and *miR20a*, were elevated after 24 hours of IL-6 treatment in both hepatoma cells and hepatocytes. Moreover, miR-18a was able to augment STAT3 transcriptional activity in HepG2 cells, which, in addition to fibrinogen protein upregulation following miR-18a transfection of IL-6 stimulated cells, highlights that a positive feedback loop may exist by which miR-18a and the STAT3 pathway, and subsequent IL-6/fibrinogen activation, upregulate one another during the acute phase response (45).

Although these data do not directly pertain to cells located in the skin, the underlying mechanisms may still be applicable however.

Table 2 – Summary of miRs involved in haemostasis during skin regeneration.

miR	Cell/Model	Role
143-145.	Mouse models (40).	Essential for VSMC* differentiation, and functionally, vasoconstriction and vasodilation.
15b.	Pulmonary artery SMCs (41).	Induced by platelet-derived growth factor, which inhibits SMC-specific gene expression and promotes cell proliferation.
221.	Pulmonary artery SMCs (42).	Induced by platelet-derived growth factor, which inhibits SMC-specific gene expression and promotes cell proliferation *via* attenuation of *c-kit*.
409-3p/29.	HuH7 (liver) cell line (43).	Attenuates Fibrinogen gene (*FGA*, *FGB*, *FGG*) expression.
18a/19a/20a.	HepG2 (liver) cell line (45).	IL-6 stimulation augmented expression of all three miRs. MiR-18a increased STAT3 activation and Fibrinogen protein level.

*VSMC = vascular smooth muscle cell

3.2. Inflammation

The inflammatory phase, as described above, depends on recruitment of immune cells to the site of the wound (46). Differentiation of blood monocytes into macrophages in the injured tissue is essential in order to mount an effective phagocytic response. This process involves a complex cascade of interlinked events in which miRs have been identified. For example, activation of the macrophage colony stimulating factor receptor (*M-CSFR*) gene due to miR-424-mediated nuclear factor I type A (*NFI-A*) transcription factor downregulation has been reported (47). Additionally, computational analysis revealed that the transcription factors CEBPB, CREB1, ELK1, NFE2L2, RUNX1, and USF2, which are involved in monocytic differentiation, target *miR-21*, *-424*, *-155*, and *-17-92* (48).

Furthermore, albeit using fibroblast-like synoviocytes isolated from rheumatoid arthritis (RA) patients, Nakamuchi et al. (49) were able to demonstrate that miR-124a impaired monocyte chemoattractant protein 1 (*MCP-1*) expression which may have significant implications for monocyte migration following skin injury.

Efferocytosis of apoptotic cells by these differentiated macrophages is critical, and has been shown to induce *miR-21*, while this miR also augmented efferocytosis, thereby demonstrating that a positive feedback loop exists. Of functional consequence, both post-efferocytotic and experimentally induced *miR-21* were able to suppress LPS-induced *NF-κB* activation and *TNF-α* expression. In addition, efferocytosis, and thus miR-21, augmented *IL-10*, which together indicates that miR-21 dampens pro-inflammatory mediators and enhances anti-inflammatory signalling (50).

TLRs enable inflammatory cells to recognise microbial pathogens, thereby aiding in the regulation of the innate inflammatory response. LPS, which is recognised by TLR4, has been shown to augment *miR-146a* and *-146b* in primary monocytes isolated from cord and adult blood (51), in addition to the monocytic cell line THP-1, whereby these miRs were shown to downregulate *IRAK1* and *TRAF6*, and thus, negatively regulate TLR signalling (52). LPS also upregulated *miR-155*.

Similarly, *miR-147* was reported to be inducible by TLR stimulation, with binding of NF-κB and STAT1α to the *miR-147* promoter also observed (53). *MiR-147* knockdown resulted in increased TNF-α and IL-6 protein concentrations, demonstrating that this miR is a significant negative regulator of TLR-induced inflammatory responses in macrophages, thereby preventing aberrant inflammation which could be causative of scar formation.

In addition to the aforementioned LPS-induced upregulation of *miR-155* (52), the same group then reported that IFN-β also augmented *miR-155* expression (54); the functional consequence of which was not identified, in contrast to the role of miR-146a. More recently however, Jablonski et al. (55) demonstrated that *miR-155*, which was upregulated in LPS and IFN-γ treated macrophages, suppresses a number of a genes, which drives the transformation to a classically activated 'M1' pro-inflammatory, anti-fibrotic macrophage phenotype *in vitro*. *MiR-155* deficient mice exhibited accelerated wound closing concomitantly with increased numbers of macrophages at the wound site following punch biopsy (56). Consistent with Jablonski et al's findings, treatment of these knockout mice with IL-4, the main cytokine involved in 'M2' or alternative macrophage phenotype activation, which is characterised by pro-fibrotic properties, induced the expression of the fibrotic protein *FIZZ1*. As expected, type-1 collagen deposition was elevated in this system. Combined, these data show that miR-155 inhibits M2 polarisation in favour of an inflammatory phenotype, and also that an overlap exists between the inflammatory phase and the growth of new tissue in the proliferation phase. MiR--155 is now also recognised as a multifunctional miR; elevated expression has been reported in activated B and T cells (57), synovial fibroblasts isolated from RA patients (58), and malignant tumours (59). Interestingly, it also regulates *BMAL1*, an intrinsic component of the circadian clock, that regulates circadian rhythm and oscillations (60).

IL-10, through activation of, and also secretion by alternatively activated 'M2' macrophages, typically possess anti-inflammatory properties. MiR-4661 transfection of RAW 264.7 macrophages resulted in augmented mRNA and protein levels of IL-10 (61). Recently, miR-142 has also been found to be critical to appropriate wound healing through the regulation of neutrophil actin cytoskeleton gene modifiers as demonstrated by *miR-142* KO mice that have abnormal wound closure rates (62).

The literature discussed above all pertains to inflammatory regulation by monocytes and macrophages; however, miRs also appear to regulate inflammatory responses in keratinocytes and in inflammatory skin disorders.

TLR2 stimulation of keratinocytes also upregulates the expression of *miR-146a*, *via* NF-κB and MAPK, with this overexpression attenuating neutrophil chemoattraction due to downregulated production of IL-8, TNF-α and CCL20, with the reverse occurring when *miR-146a* was inhibited (63). MiR-146a is also significantly associated with the inflammatory skin conditions atopic dermatitis (AD) and psoriasis. For example, *miR-146a* has been shown to be overexpressed in keratinocytes and skin lesions of AD patients (64), and functionally, appears to suppress NF-κB-dependent genes. Patients suffering from psoriasis exhibit *miR-146a* overexpression in skin lesions, which, as above, impairs neutrophil chemoattraction of keratinocytes. Genetic deficiency of *miR-146a* elicits an elevation in skin inflammation, in addition to earlier onset, due to epidermal hyperproliferation and augmented neutrophil infiltration (65). Upon administration of a miR-146a mimic, IL-17-driven inflammation was

suppressed, and the inflammatory response magnified upon inhibition, clearly demonstrating that miR-146a possesses a key role in the regulation of skin inflammation.

MiR-155 is also associated with chronic inflammatory skin disorders, including AD and vitiligo. Overexpression of *miR-155* was found in both $CD4^+$ T cells and AD patient skin samples. A positive correlation was also observed between expression of the miR and AD disease severity, Th17 cell percentage, IL-17 mRNA expression and plasma concentrations, which was further exacerbated upon transfection of a miR mimic, while an inhibitor elicited contrasting effects (66). Th17 cells have previously been postulated to significantly contribute to AD pathology, therefore it appears plausible that miR-155 contributes to disease progression due to augmentation of these cells (67). Furthermore, expression of *miR-155* was reported to be elevated in the epidermis of vitiligo patients. When overexpression was induced in primary melanocytes and keratinocytes, genes associated with melanogenesis were downregulated (68). These data indicate that miR-155 is a key regulator in the pathogenesis of the chronic skin conditions such as AD and vitiligo.

The reviews published by Rozalski et al. (68, 69) and Hawkes et al. (70) outline the extensive number of aberrations in miR expression associated with AD and psoriasis, respectively, and can be referred to for further discussion on these diseases outside the remit of this review.

Altogether the literature presented in section 3.2 demonstrates that a vast number of miRs regulate both the induction and resolution of inflammation during this stage of skin regeneration.

Table 3 – Summary of miRs involved in inflammatory phase of skin regeneration.

miR	Cell/Model	Role
424.	NB4 cell line (47).	Upregulation during monocyte/macrophage differentiation. Downregulates NFI-A transcription factor, activating *M-CSFR**.
21/424/155/17-92.	THP-1 cell line (48).	Targets of monocyte differentiation transcription factors.
124a.	Rheumatoid-arthritis fibroblast-like synoviocytes (49).	Impairs *MCP-1** expression.
21.	Monocyte-derived macrophages (human) (50).	Induces and is induced by efferocytosis. Suppresses LPS-induced NF-κB activation and *TNF-α* expression, and augments *IL-10*.
146a/146b.	Primary monocytes (cord and adult blood) (51).	Increased expression upon LPS stimulation.
	THP-1 cell line (52).	Downregulation of *IRAK1* and *TRAF6*, and thus, TLR signalling.
146a.	Keratinocytes (human) (63).	Upregulation following TLR2 stimulation and attenuated neutrophil chemoattraction.
	AD skin lesions/keratinocytes (64).	*MiR-146a* overexpression.
	Psoriasis skin lesions (65).	Overexpression in skin lesions, impairing neutrophil chemoattraction.
	KO mouse model (65).	Earlier disease onset, increased skin inflammation, epidermal hypoproliferation, augmented neutrophil infiltration. IL-17-driven inflammation suppressed upon administration of miR-146a mimic.
147.	Peritoneal/alveolar macrophages (mouse) (53).	Expression induced by TLR (LPS) stimulation. MiR-147 knockdown augmented IL-6 and TNF-α concentrations.
	RAW 264.7 cell line (53).	NF-κB and STAT1α bound to miR-147 promoter.
155.	THP-1 cell line (52).	LPS-induced overexpression.
	Primary macrophages (mouse) (54).	IFN-β-induced overexpression.
	Primary macrophages (mouse) (55).	Upregulated in LPS/IFN-γ treated macrophages. Drives transformation to pro-inflammatory 'M1' phenotype.
	KO mouse model (56).	Augmented wound closing correlated with increase number of infiltrating macrophages. IL-4 ('M2) treatment increased *FIZZ-1* expression and type 1 collagen deposition.

155.	CD4+ T cells/AD skin lesions (66).	Overexpression of *miR-155*. Expression correlated with disease severity, Th17 cell percentage, IL-17 mRNA and plasma concentrations.
	Vitiligo patient epidermis/melanocytes and keratinocytes (68).	Overexpression of *miR-155* in epidermis. Induced overexpression *in vitro* inhibited genes associated with melanogenesis.
4661.	RAW 264.7 cell line (61).	Elevated IL-10 mRNA and protein following miR transfection.
142.	KO mouse model/wound-infiltrated neutrophils (mouse) (62).	Impaired wound closure rate. Altered neutrophil phagocytosis. Actin cytoskeleton regulators *Rho* and *Rac* elevated, suggesting involvement in neutrophil migratory capacity.

*M-CSFR = macrophage colony stimulating factor receptor; MCP-1 = macrophage chemoattractant protein-1

3.3. Proliferation and Remodelling

The transition between inflammatory and proliferative phases is an essential aspect of wound healing and regeneration, and may be regulated by miR-132, which is induced by TGF-β1 and TGF-β2 in keratinocytes. MiR-132 was shown to augment keratinocyte proliferation, and similarly to miR-146a and miR-155, attenuate their chemoattractive ability *via* NF-κB suppression (71). It is likely, therefore, that miR-132 serves to mediate inflammation during progression to the proliferative phase, which is likely considering that miR-132 also suppresses NF-κB signalling.

Mobilisation of hair follicle and interfollicular epidermal stem cells during the inflammatory stage, and migration and proliferation of keratinocytes, cause skin re-epithelialisation. In an acute human skin wound model, miR-21 and miR-130a have been reported to delay re-epithelialisation (72). Conversely however, TGF-β1-induced *miR-21* expression was able to augment keratinocyte migration in HaCaT cells (73). Consistently, knockdown of *miR-21* decreased TGF-β1-induced keratinocyte migration. Following mouse skin punch biopsies, *miR-21* expression was elevated, while *miR-21* knockdown impaired re-epithelialisation. Both *in vitro* and *in vivo* data from this study strongly suggest that miR-21 drives keratinocyte migration and proliferation. The data regarding miR-21 appear somewhat conflicting, however, these differences may be due to the use of human cell lines and mouse skin model in one investigation (73), and the use of an acute human skin wound model in the other (72). Nonetheless, it is clear that miR-21 possesses a significant regulatory role in keratinocyte migration and thus, re-epithelialisation.

In addition to the results reported by Yang et al. (73), Li et al. (74) showed that another transforming growth factor, TGF-β2, known to be highly expressed in skin wounds, elicits a significant elevation in the expression of *miR-31* and subsequent proliferation in primary human keratinocytes, with *miR-31* knockdown causing contrasting effects. Interestingly, EMP-1 appeared to mediate the effects of miR-31; a significant negative association was observed between the two, while silencing of *EMP-1* exerted similar effects as *miR-31* overexpression in terms of migratory capacity. *In* vivo, punch biopsy of a human wound healing model

demonstrated that *miR-31* gradually increased from the first day, and thus, the inflammatory phase, until day seven during the proliferative phase (74). This not only supports the postulation that considerable overlap exists between the phases of skin regeneration, but also that miR-31 regulates re-epithelialisation in a similar manner to miR-21.

An inverse correlation between *RAN* and *RAPH1*, and *miR-203* expression was observed (75). These two proteins, involved in cell proliferation and survival, and cytoskeleton remodelling respectively, have shown to be direct targets of miR-203. Furthermore, both silencing of these targets and overexpression of *miR-203 in vitro* using human neonatal epidermal keratinocytes resulted in attenuated cell proliferation and migratory capacity. *In vivo*, elevated *miR-203* expression was reported in the suprabasal epidermal layers surrounding the wound in a mouse skin model, however, minimal expression was found in the migrating keratinocytes themselves. MiR-203, could, therefore, be a possible target for therapeutic intervention, given the need for keratinocyte migration and proliferation in re-epithelialisation, and the evident role miR-203 possesses in terms of inhibiting these key processes.

Reactive oxygen species released by phagocytic cells during the inflammatory phase appear to drive angiogenesis as evidenced by H_2O_2-induced *VEGF* augmentation in keratinocytes (76), in addition to impairment of angiogenesis upon antioxidant treatment in human microvascular endothelial cells (77). In the study conducted by Shilo and colleagues (77), despite elevated *VEGF* expression following *Dicer* knockdown, the angiogenic response of these endothelial cells was compromised, demonstrated by attenuated tube formation and cell migration. Furthermore, *Dicer* knockdown in human endothelial cells elicited aberrant expression of a number of angiogenic genes concomitantly with attenuated cell proliferation (78). Similarly, mice that lacked the first two exons of the *Dicer* gene exhibited under-developed blood vessels (79).

Moreover, miR-221 and -222 transfection of endothelial cells results in a reduction in c-Kit protein levels through targeting the c-Kit 3'UTR (80). c-Kit signalling involves Akt and Erk1/2 pathways, similarly to VEGF (81), while activation of c-Kit has been shown to also upregulate *VEGF* (82). Thus, miR-221 and -222 appear to regulate angiogenesis directly through c-Kit, which itself is the receptor for stem cell factor, and indirectly *via* c-Kit-dependent modulation of VEGF. Tissue hypoxia due to reduced blood supply of the damaged skin is also known to be an inducer of angiogenesis during regeneration; *ETS-1,* the angiogenesis-related transcription factor, in addition to *MMP1 and VEGFR1*, are negatively regulated by miR-200b. Expression of *ETS-1* was de-repressed following hypoxia-induced downregulation of *miR-200b*, which augmented angiogenic capacity (83), again, in experiments that utilised endothelial cells.

Seven days post-skin excision injury in mice, over 50 miRs exhibited altered expression of greater than two-fold, 33 of which were upregulated and 21 downregulated. The former included *miR-21*, *-31*, and *-203*, and the latter, *miR-249*. Prior to skin injury, *miR-21* expression was undetectable in the epidermis, however, following excision, expression in the migrating epithelial cells was augmented greatly (84). Furthermore, mesenchymal expression of *miR-21* was also elevated in granulation tissue. Overexpression of *miR-21* has been shown to inhibit granulation tissue formation in a rat wound model (72), which could have significant implications for therapeutic targeting in chronic non-healing wounds. Interestingly, TGF-β

signalling has been robustly shown to not only induce *miR-21* expression in keratinocytes (85), but is also a key pathway involved in the contraction of wounds (86).

In terms of collagen deposition, augmented *miR-29a* expression was shown to occur concurrently with a reduction in collagen type 1 alpha 2 (*COL1α2*) and *VEGF-A* following thermal skin injury (87). As *miR-29a* began to decrease, *COL1α2* and *VEGF-A* began to increase, suggesting that they were targets of miR-29a, which was discovered to be the case. This was further demonstrated by inhibition of *miR-29a*, which elicited a significant elevation in fibroblast proliferation and migration. The naturally occurring downregulation of *miR-29a* during skin regeneration, therefore, appears to be a mechanism by which type 1 collagen synthesis and angiogenesis are enhanced, aiding the regenerative process. In a similar manner, Zhu et al. (88) corroborated these findings by recently reporting that *miR-29a* exhibited downregulation in a murine thermal wound model, however, much more drastic attenuation of *miR-29b* was observed in both thermal and excisional wound models, concomitantly with a significant elevation in heat shock protein 47 (*HSP47*) expression and a gradual increase in *COL1α1*. Of note was that TGF-β1 inhibited *miR-29b* transcription in skin fibroblasts; given that *miR-29b* overexpression impairs biosynthesis of COL1α1, this further highlights the important of TGF-β signalling in collagen deposition. Together, these data demonstrate that the miR-29 family possesses essential regulatory roles in mediating fibrotic processes and collagen deposition associated with wound contraction and scar formation. It has long been suggested that miR-29 is "fibromiR" and is a critical target in fibrotic diseases where constitution of miR-29 is currently being investigated; the miR-29 family shares seed sequences complementary to conserved binding sites of multiple collagen genes (89) and downregulation of *miR-29a* in SSc, a major fibrotic disease, has also been shown (90); experimentally, this study reported that overexpression of *miR-29a* caused a decrease in type I and type III collagen mRNA, with the opposite effects observed upon *miR-29a* knockdown. These data were corroborated, as increased *miR-29a* expression reversed the fibrotic SSc fibroblast phenotype due to attenuation of collagen and TIMP-1, which itself is regulated by the miR-29a target TAB1 (91).

MiR-145 appears to regulate a similar cascade; i*n vivo*, *miR-145* levels and α-smooth muscle actin (*α-SMA*) were significantly augmented in hypertrophic skin tissue compared with controls, while TGF-β1-induced elevation of *miR-145* attenuated expression of *KLF4*, thereby de-repressing *α-SMA* in skin myofibroblasts (92). Thus, aberrant *miR-145* appears to contribute to scarring due to α-SMA's contribution to permanent tissue contracture. Furthermore, inhibition of miR-145 attenuated not only *COL1α1* expression, but also TGF-β1 secretion, and migration.

A reduction in miR-129-5p also appears to possess a significant regulatory role in aberrant fibrotic processes associated with SSc pathology; *in vitro*, IL-17RA siRNA transfection significantly reduced expression of the antifibrotic *miR-129-5p*. Moreover, TGF-β1-induced downregulation of IL-17A signalling due to attenuation of the receptor *IL-17RA* expression in SSc fibroblasts releases the inhibitory effect of miR-129-5p on type 1 collagen, thereby promoting fibrosis (93).

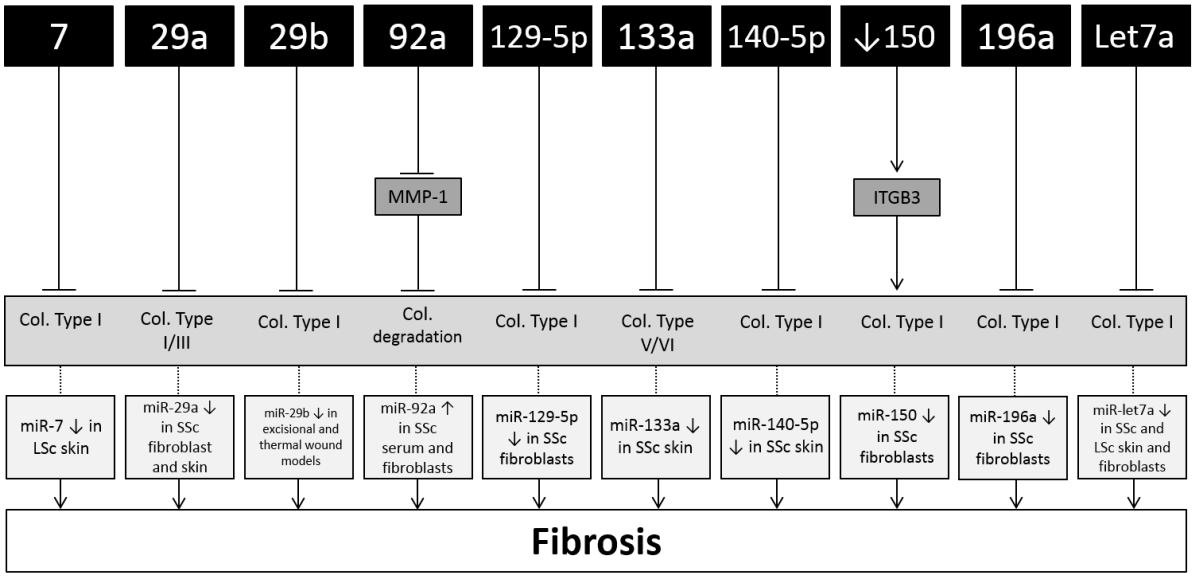

Figure 3 – Schematic representing miRs involved in collagen regulation and scleroderma pathogenesis. MMP-1 = matrix metalloproteinase-1; ITGB3 = integrin beta 3; Col. = collagen. References: (88, 90, 91, 93-99).

Table 4 – Summary of miRs involved in proliferation and remodelling phase of skin regeneration.

A paucity of literature appears to exist concerning miR-mediated longer term remodelling of the skin, whereby type III collagen is replaced by type I collagen and re-aligned. This area, therefore, warrants further investigation so that the molecular mechanisms that govern wound healing are fully characterised.

miR	Cell/Model	Role
132.	Keratinocytes (human) (71).	Augments proliferation and attenuate chemoattractive ability.
21.	Skin punch biopsy (human/rat) (72).	Inhibits re-epithelisation.
	HaCaT cell line (73).	TGF-β1-induced *miR-21* expression increased keratinocyte migration.
	Excisional wound model (mouse) (84).	Elevated expression in migrating epithelial cells following skin injury.
	HaCaT cell line (85).	TGF-β signalling induced *miR-21* expression. MiR-21 blocks inhibition of growth by TGF-β1.
130a.	Healthy skin punch biopsy (human) (72).	Inhibits re-epithelisation.
31.	Keratinocytes (human) (74).	Expression induced by TGF-β2
	Skin punch biopsy (human) (74).	Expression gradually increased from day 1 until day 7.
203.	HEKn cells (75).	Overexpression attenuated cell proliferation and migratory capacity.
	Skin punch biopsy (mouse) (75).	Expression found in suprabasal epidermal layers. Minimal expression in migrating keratinocytes.
221/222.	Endothelial cells (human vascular) (80).	Transfection reduces c-Kit, thereby reducing angiogenesis.
200b.	Dermal microvascular endothelial cells (human) (83).	De-repression of angiogenic transcription factor *ETS-1*, and thus increased angiogenesis, following hypoxia-induced *miR-200b* downregulation.
29a.	Thermal wound injury model (rat)/BJ fibroblast cell line (human) (87).	Augmented expression concurrently with attenuated *COL1α2* and *VEGF-A* (confirmed as targets of miR-29a *in vitro*).
	Excisional and thermal wound models (mouse) (88).	Expression unaltered in excisional wound model, but downregulated in thermal wound model.
	NRK-52E cell line (89).	3'-UTR of numerous collagen genes targeted by miR-29 family.
	SSc fibroblasts/skin sections (human) (90).	Downregulation in SSc fibroblasts and skin sections. Overexpression of *miR-29a* caused a decrease in type I and type III collagen mRNA, with the opposite effects observed upon *miR-29a* knockdown.
	Dermal fibroblasts (human) (91).	Transfection of miR-29a decreased collagen and TIMP-1. TAB-1, a regulator of TIMP-1, found to be a novel target of miR-29a.

29b.	Excisional and thermal wound models (mouse) (88).	Downregulation in both models. Transcription inhibited by TGF-β1, enhancing collagen 1 production.
145.	Hypertrophic scar tissue (human) (92).	*α-SMA* and *miR-145* elevated.
	Dermal fibroblasts (human) (92).	Expression augmented by TGF-β1, causing a decreasing in *KLF4*, thereby de-repressing *α-SMA.*
129-5p.	SSc patient skin and serum samples (93).	IL-17A transfection attenuated *miR-129-5p* expression. Downregulation of IL-17A signalling by TGF-β1 releases inhibitory effect of miR-129-5p on collagen type 1.

3.4. Foetal Wound Healing

Although not as common as tissue injuries in adults, foetal skin possesses the capacity to heal without scarring due to its own unique molecular response to injury. Microarray analysis has revealed a plethora of differentially expressed genes between mid-gestational and post-natal dermal wounds, concomitantly with the expected full re-epithelialisation and lack of scarring in the former, and dense scar tissue present in the latter mice (100). Most importantly, a number of these differentially regulated genes were involved in growth factor signalling and cell proliferation. Further support for the significance of growth factors in the striking phenotypic differences between early-mid foetal skin and late foetal or post-natal skin has been reported. Unwounded human adult and foetal skin possess distinct *TGF-β* expression profiles, with lower expression of all three isoforms found in foetal skin, in addition to differential ratios compared with adult skin (101). Given the role of TGF-β in the regulation of extracellular matrix deposition, it was unsurprising that deletion of *TBF-βRII* significantly attenuated dermal scar formation and enhanced epidermal proliferation in post-natal fibroblasts (102). The dermis of *TBF-βRII* knockout mice also exhibited a decrease in collagen deposition together with augmented keratinocyte proliferation, and thus, re-epithelialisation (86).

In addition to growth factors, aberrant collagen composition and organisation also contribute to the scarring phenotype which is not characteristic of non-scarring foetal skin. Mid-gestational rats exhibited a much higher collagen type III:collagen type I ratio than adult rats following surgical dermal injury (103). In corroboration, Goldberg et al. (104) discovered that *COL1α1* expression was attenuated following injury during mid-gestation only, while *COL1α2 COL1α3* were significantly lower in the late-gestational group, in both wounded and normal conditions. The pattern of collagen deposition in the mid-gestational mice was characteristic of the surrounding unwounded skin with no visual evidence of scarring, whereas irregular collagen deposition in the late-gestational mice was observed, suggesting scar tissue formation. *In vivo*, undamaged human foetal skin also exhibited a greater type III:type I ratio than adolescent, adult, and elderly skin (105).

A comparison of mid-gestational (non-scarring phenotype) and late-gestational (scarring phenotype) mouse skin revealed a number of differentially expressed miRs and predicted targets between the two time-periods and associated phenotypes. In particular, expression of *miR-29b, -29c*, and *338-3p* was altered 24, 20, and 19-fold, respectively (106). Importantly,

bioinformatic analysis revealed that the differentially expressed miRs were also shown to putatively target a number of signalling pathways, including TGF-β. Thus, it could be postulated that miRs may be significant regulators of the foetal non-scarring phenotype due to modulation of TGF-β signalling. The role of the miR-29 family in the regulation of collagen expression has already been discussed within this review, and may present a further molecular mechanism by which differential miR expression contributes to scarless healing.

4. MicroRNA Therapeutics

In addition to the essential role of miRs in normal development of the skin and regeneration from injury, they also play a pivotal role in the pathogenesis of a vast number of diseases, including conditions of the skin such as vitiligo, psoriasis, SSc, dermatomyositis, and melanomas. The detection of miRs in serum or plasma, which resist degradation due to containment within extracellular vesicles, are emerging as non-invasive diagnostic markers for major diseases like cancer (107) and cardiovascular disease (108), in addition to rheumatoid arthritis (109), SSc (110), and as a biomarker of the severity of inflammation in children with AD (111).

In terms of treatment of specific conditions, recent research has demonstrated that miRs can manipulated *via* administration of miR mimics which are chemically synthesized, double-stranded RNAs that mimic endogenous miRNAs, while antagomiRs are chemically engineered oligonucleotides that inhibit miRNAs. There is potential for both to be used therapeutically for tissue regeneration or fibrotic diseases. As highlighted by Christopher et al. (112), however, a number of steps are required before an miR could potentially be used as an effective therapy, namely; profiling of the miR associated with a specific disease state, *in vitro* studies to validate the miR using loss/gain of function, *in vivo* studies to investigate pharmacokinetics, followed by clinical trials if all other stages are successful. Other important considerations are specificity of binding to the target miR, resistance to degradation, and the method of *in vivo* delivery.

Chemical modification of the miRs are required to enhanced stability and stop the breakdown by copious endogenous nucleases, in addition to improving affinity of the antagomiR to the cognate miR. Such examples are Locked Nucleic Acids (LNAs), whereby the ribose of the RNA nucleotide is chemically modified by the addition of a 2'-O, 4'-C-methylene bridge (113). Alternatively, tiny LNAs which are 8-mer LNA anti-miRs that specifically target the miR seed region, can be used (114). Chemical substitution of the 2' hydroxyl group, to 2'O-methyl or 2'O-methoxyethyl, for example, also occur (115).

The issue of specificity is being addressed *via* a number of methods; sponge miRs, for example, contain several complementary binding sites to the specific miR of interest, whereas miR erasers utilise two copies of the exact miR complementary antisense sequence (116). Long non coding RNAs (lncRNA) are longer forms of RNA arbitrarily defined as 200nts and over, and often act as sponges to sequester faulty miR expression. Indeed, the X-linked lncRNA H19 is aberrantly expressed in keloids (117). Targeting of the miR to the correct tissue could also be employed by conjugating the chemically synthesised miR to a monoclonal antibody that identifies that specific tissue antigen, thereby 'hitting the target' and causing the miR to bind to its cognate mRNA. This could also be PEGylated to increase stability.

Viral vectors have been utilised in order to deliver miR mimics or inhibitors. For example, adeno-associated virus (AAV) is a popular gene delivery system; however, a consistent issue which has inhibited the progression of some AAV therapies to human clinical trials is the induction of low grade immune activation (118). Lenti-viral vectors for delivery suffer from the same immunogenicity issues. MiR mimics for the tumour suppressors miR-34a and let-7 have been successfully delivered in a complex with neutral lipid emulsion in mice (119).

A number of miR therapies are currently in preclinical trials, with a small number having already met the requirements to advance to clinical studies. Although no miR therapies designed specifically for skin disorders are at this stage to the authors' knowledge, MiRagen

Therapeutics currently have an ongoing phase 1 clinical trial for MRG-201, a miR-29b mimic which is designed to attenuate collagen expression, as this is a true target of miR-29. A number of studies have also utilised miR mimics and antagomiRs *in vitro*, and could potentially inform future studies which may progress to clinical trials.

Loss/gain of function studies are essential in order to validate particular miRs that may be future therapeutic targets with regard to skin disorders and/or healing. MiR mimics and inhibitors have shown that *miR-200b* expression was up- and down-regulated, respectively, in human microvascular endothelial cells, with a subsequent attenuation in tube formation and wound closure following mimic administration, with contrasting effects observed following addition of the 200b inhibitor (83). In a similar investigation, the addition of a miR-29b mimic to primary human dermal fibroblasts elicited a significant augmentation of *miR-29b* expression with a concomitant attenuation of *HSP47* and *COL1α1* expression. As expected, contrasting outcomes were observed following administration of an inhibitor (88). These miRs could, therefore, be future targets for therapeutic intervention. Furthermore, due to the data presented above by Gras and colleagues (92), they concluded that miR-145 may also be a promising target for future therapeutic intervention.

With specific regard to the utilisation of antagomiRs, Krützfeldt et al. (120) have demonstrated that intravenous injection of antagomiRs against miR-16, -122, -192, and -194, resulted in a subsequent attenuation of the miR levels in a number of major tissues, including the skin. Subcutaneous injection of a miR-203 antagomiR attenuated *miR-203* expression, but importantly, resulted in a greater number of proliferative cells in the dorsal epidermis of neonatal mice (23). Similarly, in a mouse excisional wound model, injection of an antagomiR to miR-155 attenuated expression as expected, in addition to phagocytic cell migration, pro-inflammatory cytokine secretion, and *COL1α1*, *COL2α1*, and *α-SMA* expression. Functionally, this elicited an overall positive effect as demonstrated by better aligned and thinner collagen fibres upon wound healing (121). Most recently, topical epicutaneous administration of the miR-155 antagomiR elicited a reduction in collagen deposition and dermal thickening in bleomycin-induced fibrotic mice (122). Of greatest interest, however, was that due to the topical delivery method, miR-155 was downregulated only in the skin, and not liver, bone marrow, or blood cells.

Direct injection of a miR-21 antagomiR to the dermis surrounding a would site did indeed attenuate expression of *miR-21*, however impaired collagen deposition and delayed wound healing were observed which the authors did not expect, and therefore, miR-21 may not be an effective therapeutic target for wound healing (84).

Another issue is that targeting one specific miR might not be sufficient to elicit a significant clinical effect due to large redundancy among miRs; a reduction in one miR may have negligible effects on the protein output due to one or more miRs compensating for this. Thus, it may take multi-miR targeting approaches to repress a specific pathway.

Whilst hematopoietic stem cell transplantation (HSCT) isn't an miR-based therapy *per se*, we hypothesize that miRs could be reset to non-aberrant levels thereby attenuating *IL-6*, *TNF-R*, and CD3+ cell numbers, which was indeed observed in the dermis of the patient presented in figure 4 (123). Concomitantly, restoration of the skin structure also occurred, which would be expected given the role of IL-6 in promoting fibrosis (124). It must be noted that no current data exists to support or refute this postulation however.

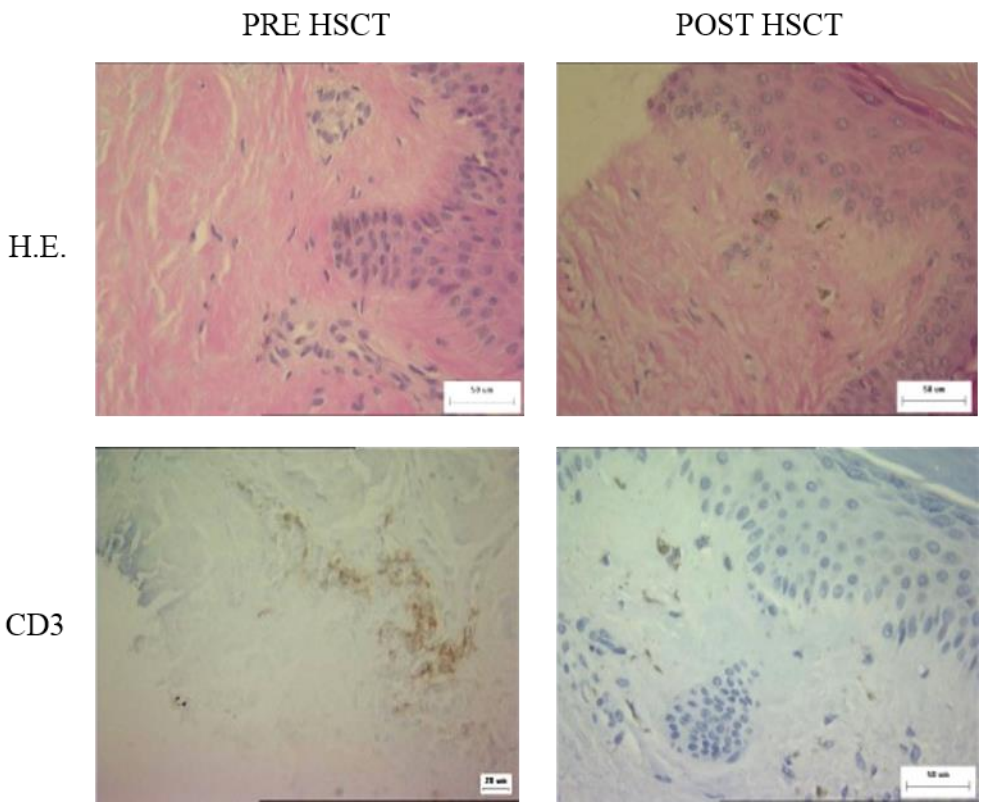

Figure 4 – Hematoxylin and eosin (H.E.) and T cell (CD3) immunohistochemistry staining of a SSc patient's dermis, before and after HSCT.

5. Concluding Remarks and Future Directions

Although originally referred to as 'junk DNA' with little to no biological function, it has become increasingly apparent that non-protein coding regions of the genome can still exert a multitude of diverse effects and are no longer deemed insignificant. Non-coding RNA are one such example that mediate a vast number of functions *via* post-transcriptional gene regulation, in the case of miRs (125).

The skin and the underlying molecular mechanisms by which it develops, is maintained, and regenerated, while complex, has been reasonably well characterised at this point. Of interest however, is how these mechanisms can be manipulated in order to elicit salubrious outcomes in the context of wound healing or skin disorders such as scleroderma. Persistent perpetuation of dysregulated miRs in skin disorders suggest they are key players in disease progression, and may also, therefore, be promising biomarkers and/or therapeutic targets. Data thus far has highlighted a variety of potential therapeutic targets *in vitro* and using murine models, however, as discussed, a large number of stages and considerations are required prior to a specific miR becoming eligible for clinical trial. Further research should continue to identify and validate miRs associated with aberrant epidermal development and homeostasis associated with disease in the hope that efficient miR-based therapies may be established. It may be possible in the future with the advent of viral gene delivery to alter the expression of miRs with the use of viral vectors providing immune activation is avoided. In particular SSc may be a tractable target for miR viral gene delivery due to the wealth of knowledge of miRs in this condition.

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